Supplemental Information

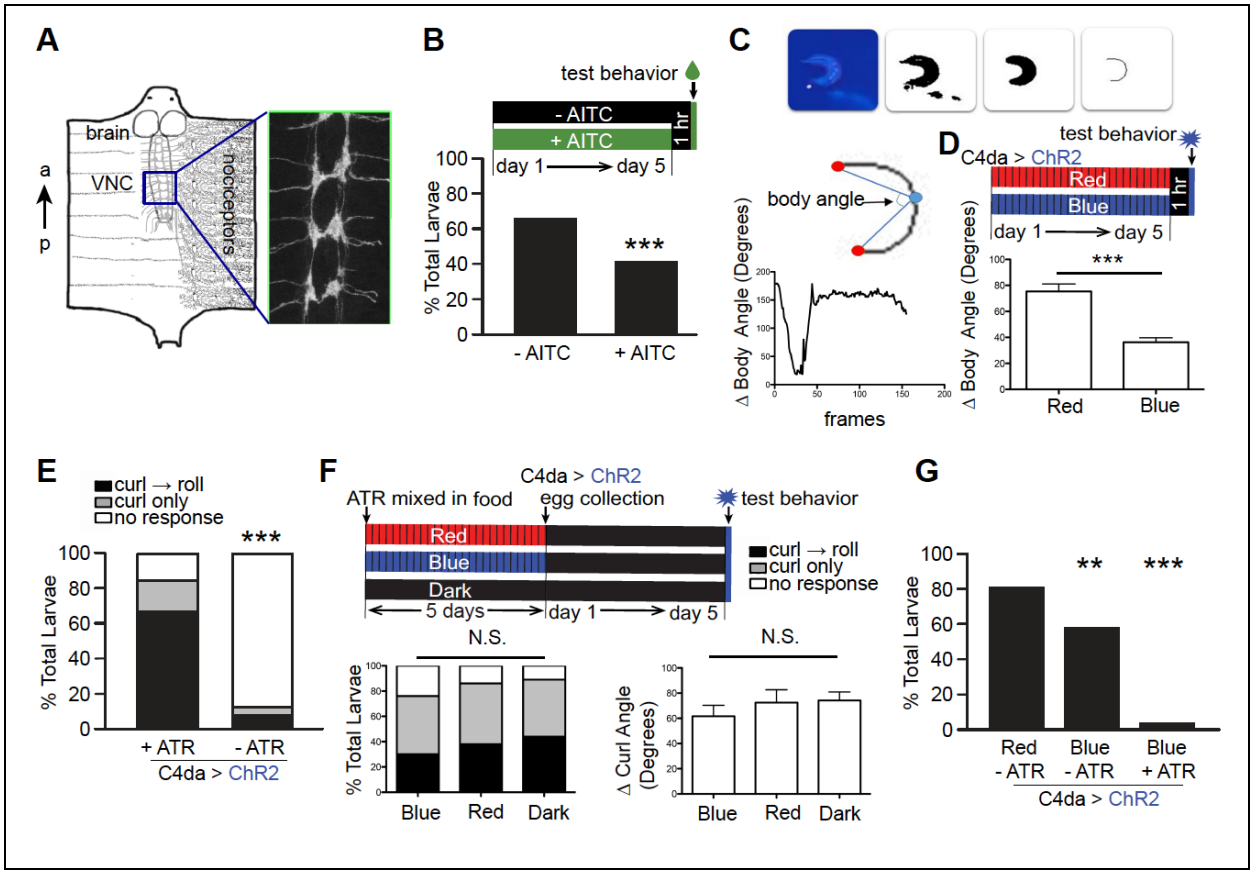
Serotonergic modulation enables pathway-specific plasticity in a developing sensory circuit in *Drosophila*

Takuya Kaneko, Ann Marie Macara, Ruonan Li, Yujia Hu, Kenichi Iwasaki, Zane Dunnings, Ethan Firestone, Shawn Horvatic, Ananya Guntur, Orie T. Shafer, Chung-Hui Yang, Jie Zhou, and Bing Ye

Inventory of Supplemental Information

Supplemental Data

Supplemental Figure Legends



Supplemental Figure 1 (Related to Figure 1). Developmental stimulation of nociceptors suppresses nociceptive behavior.

(**A**) Schematic of a larval fillet used for live-imaging studies. “a” and “p” indicate anterior and posterior of the larval fillet, respectively. The axon terminals of C4da neurons in several segments are magnified.

(**B**) Raising larvae on AITC (5 mM) leads to suppression of nociceptive behavior. The behavioral tests were performed with AITC on 3rd instar larvae. The graph indicates the percentage of larvae showing a complete (i.e. 360˚) rolling within 2 min in response to 25 mM AITC. *n* = 440 and 124 larvae for – AITC and + AITC, respectively.

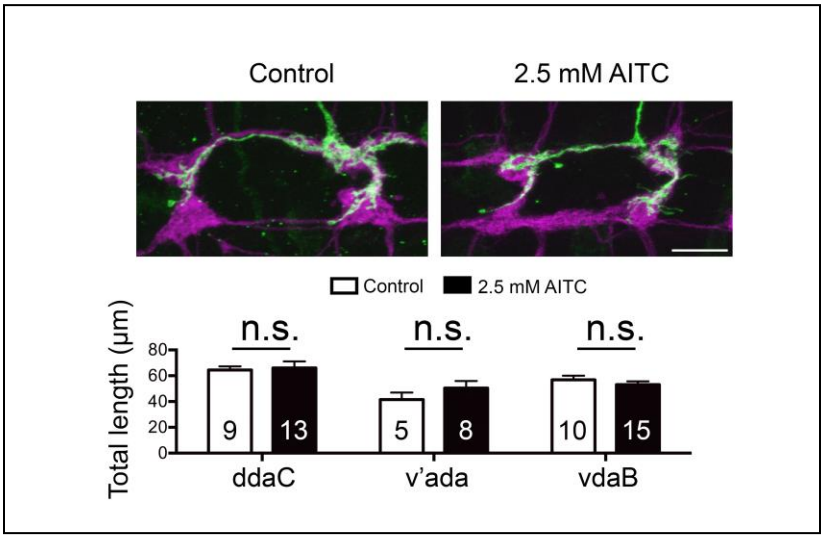
(**C**) Diagram illustrating the automatic measurement of larval body angle to quantify curling behavior. A single larva was extracted from the video-recording to obtain its skeleton using automatic image processing. The two red dots indicate the end points of the skeleton line, and the blue dot is the middle point of the line. Graph indicates body angles of larvae during curling in response to ChR2-mediated stimulation. The speed is 30 frames/sec.

(**D**) Optogenetic activation of C4da neurons during development results in a significant reduction in curling (*n* = 70 larvae for each group).

(**E**) All-trans-retinal (ATR) is required for ChR2 activation of C4da neurons (*n* = 115 larvae for each group). Larvae were developed in constant darkness for 5 days in food with or without ATR. Larvae developed without ATR (right) do not exhibit robust responses to blue light compared to larvae with ATR (left).

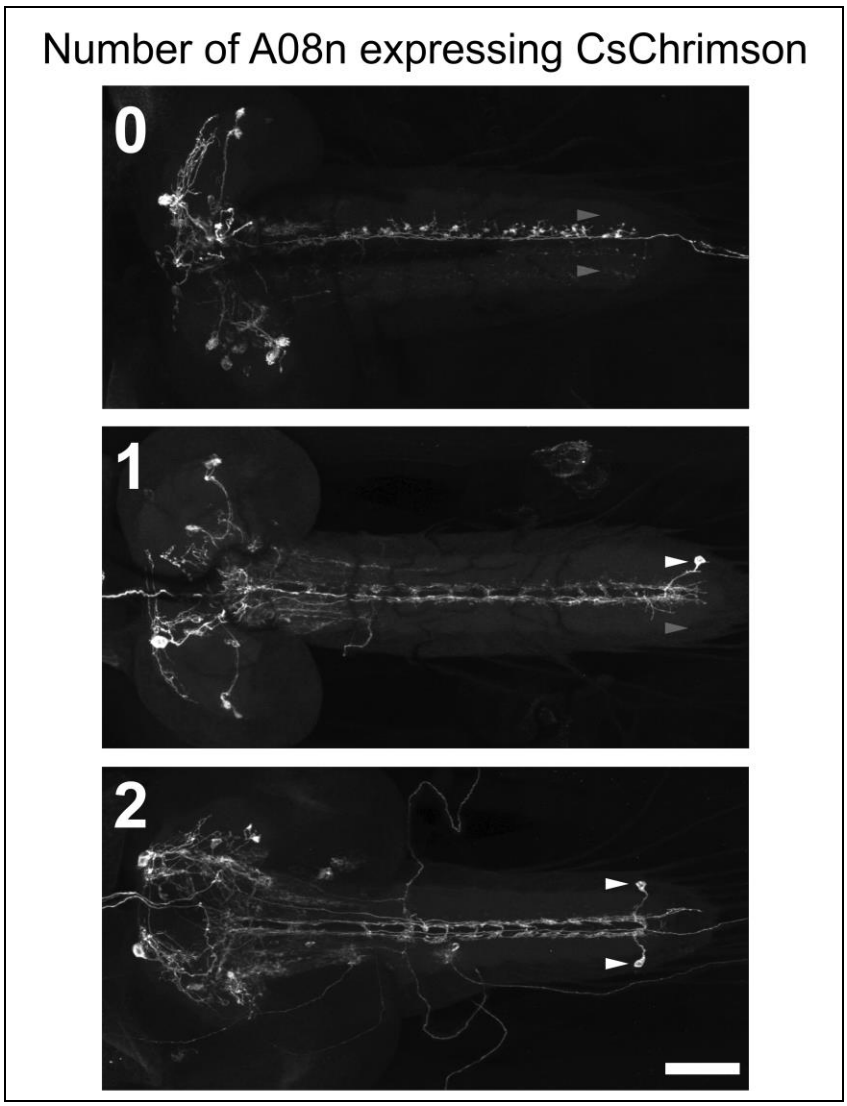
(**F**) ChR2-mediated suppression of nociceptive behavior is not due to ATR bleaching. 4 mM ATR-containing food was kept under blue or red light or constant darkness for 5 days. Larvae were then allowed to develop on food in constant darkness for 5 days before being tested for behavior. No change in nociceptive behavior was observed between the tested conditions, as

analyzed using Kruskal-Wallis tests. *n* = 30, 30 and 20 larvae for blue, red and dark, respectively, for curling angle analysis; *n* = 60, 60 and 45 larvae for blue, red and dark, respectively, for percent of total larvae that exhibited nociceptive responses.
(**G**) Illumination with blue light alone (without ATR) during development led to a mild decrease in nociceptive rolling. The behavior test was performed with 100 mM AITC. *n* = 58, 59, and 83 larvae for Red – ATR, Blue – ATR, and Blue + ATR, respectively.

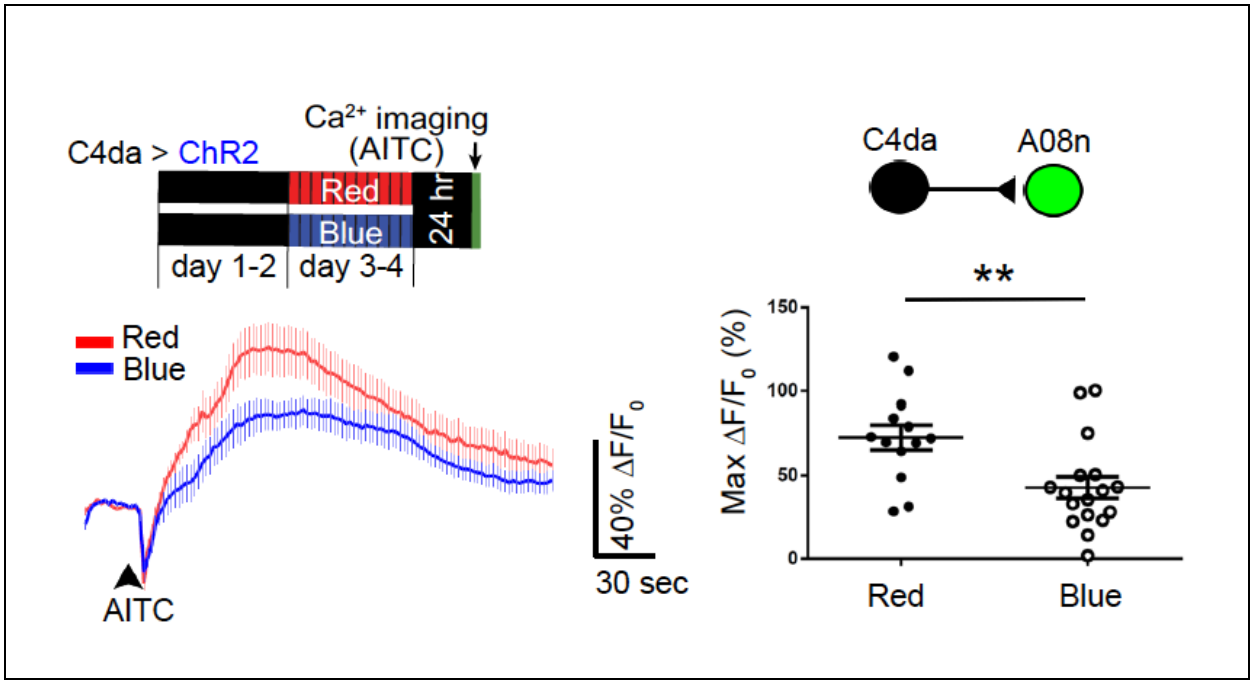


Supplemental Figure 2 (Related to Figure 1). Developmental activation of C4da nociceptors with AITC does not change the size or targeting of their presynaptic terminals.

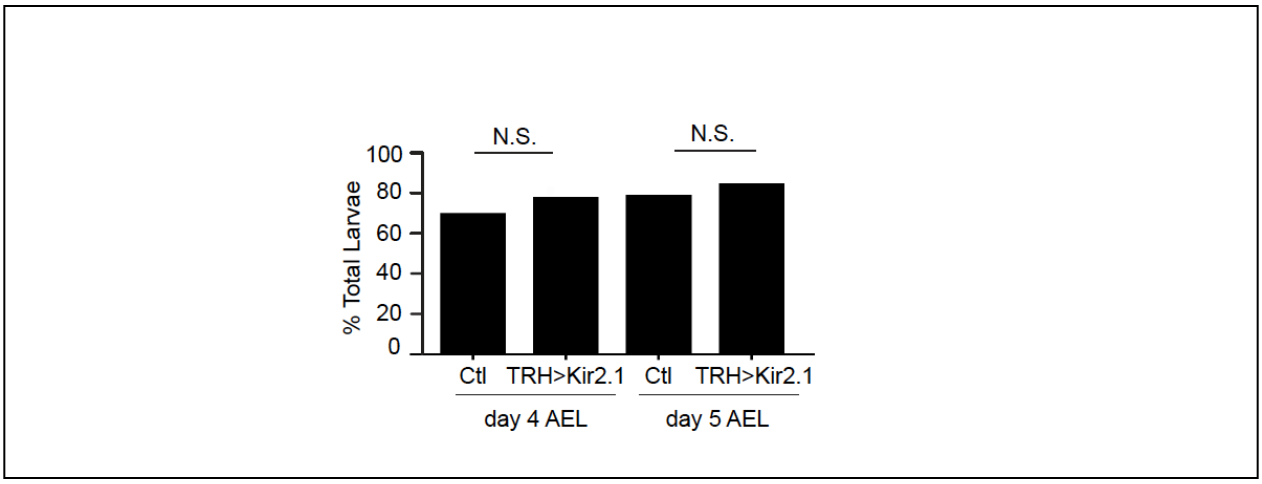
Micrographs show the presynaptic terminal of a representative ddaC neuron (green) from each group. Presynaptic terminals of the other C4da neurons in the same body segment were labeled using a different fluorescent protein (magenta). Quantification of each subtype of C4da neurons (ddaC, v'ada, and vdaB) is shown in the bar chart.



Supplemental Figure 3 (Related to Figure 2). Mosaic activation of A08n neurons. CsChrimson::Venus was expressed in none ("0"), one ("1"), or both ("2") A08n neurons, in addition to several neurons in the brain. The FLP-out mosaic technique with GMR82E12-Gal4 driver was used for the mosaic expression of CsChrimson. Arrowheads point to the cell bodies of A08n. Scale bar: 50 µm.

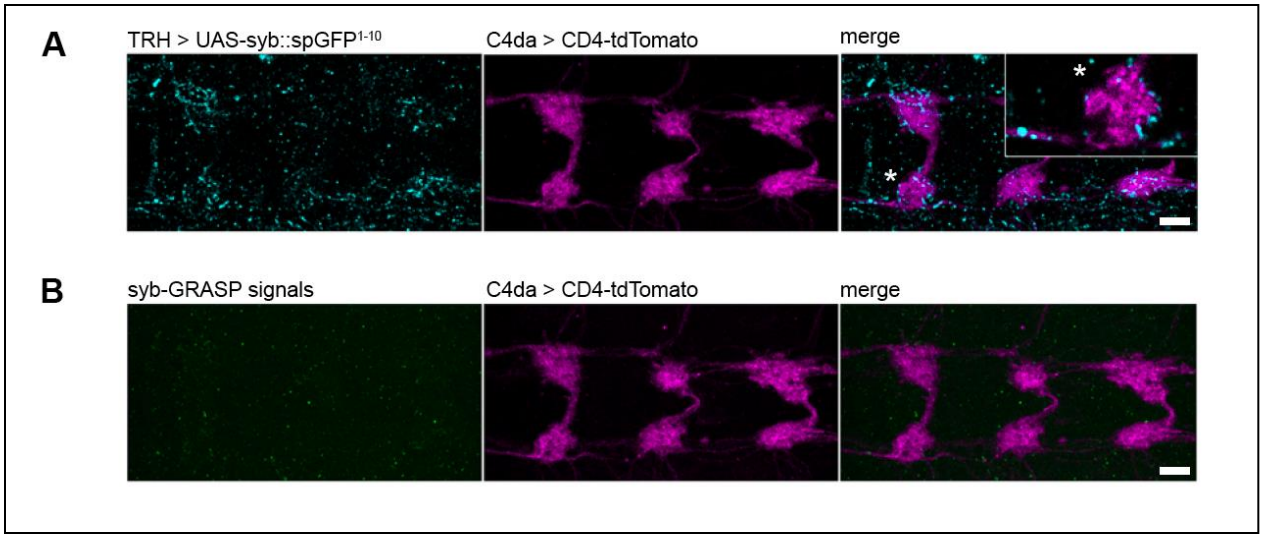


Supplemental Figure 4 (Related to Figure 3). High levels of nociceptive inputs during development suppress C4da-to-A08n synaptic transmission.
Nociceptor activation during day 3-4 AEL of larval development resulted in reduced A08n responses. *n* = 13 neurons (7 larvae) and 17 neurons (10 larvae) in red and blue groups, respectively.



Supplemental Figure 5 (Related to Figure 4). Silencing serotonergic neurons does not affect the nociceptive behavioral responses if nociceptors are not stimulated during development.

Kir2.1 was expressed in serotonergic neurons to silence these neurons (TRH>Kir2.1). The negative control (Ctl) was UAS-Kir2.1 alone without the TRH-GAL4 driver. 25 mM AITC was used to assess larval rolling behavior on day 4 AEL (*n* = 106 larvae for Ctl and 72 for TRH>Kir2.1) and day 5 AEL (*n* = 57 for Ctl and 59 for TRH>Kir2.1). The percentage of larvae showing a complete rolling within 2 min was quantified. No significant difference was observed between the two groups. Larvae younger than day 4 AEL were not tested because they rarely exhibit nociceptive rolling (data not shown).



Supplemental Figure 6 (Related to Figure 5). The terminals of serotonergic neurons intimately intertwine with C4da presynaptic terminals.

(**A**) Terminals of TRH neurons expressing the presynaptic marker, syb-spGFP$^{1-10}$ (cyan), near C4da neuropils (magenta). The inset in "merge" shows one C4da neuropil (marked with "*") in a single focal plane. Scale bar: 5 µm.

(**B**) No syb-GRASP signal was observed from TRH terminals to C4da presynaptic terminals. Syb-spGFP$^{1-10}$ was expressed in TRH neurons; CD4-tdTomato and CD4-spGFP^{11} were expressed in C4da neurons. Scale bar: 5 µm.